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Antagonistic Effects of Suramin Against the Venom of the Iranian Snake *Echis carinatus* in Mice

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Abstract

Echis carinatus (Saw-scale viper) is one of the most venomous snakes in Asia and some parts of the Africa with deadly hemotoxic venom. It has been reported that suramin, an anti-trypansomiasis drug, can inhibits the toxic effects of some snake venoms. This study was conducted to evaluate the antagonistic effects of suramin against the venom of the Iranian snake *Echis carinatus*.

Adult albino mice weighing 35±5g were divided into nine groups (A_1, A_2, B_1, B_2, B_3, C_1, C_2, D and E) of 6-10 mice. Groups A_1 and A_2 (protocol A), which received the venom at 7 and 13 mg/kg, succumbed after 169±32 min and 53±16 min, respectively. Groups B_1, B_2 and B_3 (protocol B) were treated with different doses of suramin 15 min after injection of venom. The results show that suramin significantly delayed time to death in groups B_1, B_2 and B_3 compared with groups A_1 and A_2 ($P<0.05$). In groups C_1 and C_2 (Protocol C), venom was pre-incubated with suramin for 15 min prior to injection into animals. The survival times of these two groups were significantly increased when compared to group A_2. Hhowever, only group C_1 in comparison with group A_1 had a significantly increased survival time ($P<0.05$). Group D (protocol D) was treated with suramin 15 min before injection of venom. Time to death in this group increased in comparison to group A_2. Group E received suramin alone as a control, and all the animals in this group remained alive. The results of this study showed that a single dose of suramin have a protective role against *Echis carinatus* venom at least in delaying time to death of envenomed animals. Repeated administration or higher doses of suramin may be able to prevent death caused by the venom.

Keywords: suramin, *Echis Carinatus*, venom, antivenom, antagonist

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Introduction

Snake bites can be fatal if not treated quickly. Snake venoms with haematoxic effects are rich in different factors including a variety of proteins and peptides that affect the haemostatic system (Marsh, 1994, Backshall, 2007). In general, the venom proteins of the vipers family (Vipera) affect blood clotting factors by first starting the clotting process and then blocking formation of larger clots, and also by damaging the artery walls leading to non-stop profuse bleeding (Warrell *et al.*, 1977, Mallow *et al.*, 2003). The venom of *Echis carinatus* (Fig. 1) reduces most clotting factors including V, VIII, II and XIII and, by direct activation of prothrombin, affects blood coagulation that in turn causes haemostatic defect (Warrell *et al.*, 1997, Ali *et al.*, 2004).

Echis carinatus is generally considered to be one of the most deadly snakes in the world. It is believed that this viper could be responsible for more human deaths than cobras, mambas and rattlesnakes together (Warrell *et al.*, 1977, Phelps, 1981, Backshall, 2007). Although there is little definitive information on the incidence of bites or total fatality rate due to *Echis carinatus* in Iran, this snake is certainly responsible for many envenoming bites in this country. In one report on 103 envenomations in the south of Iran, *Echis carinatus* was one of the major causes of envenoming (Emam and Nikzamir, 2008).

In general, like other snakebites, the conventional treatment for envenomation by this snake is administration of anti-snake venom (ASV) soon after bite. Anti-snake venom administration would be less effective if the time from envenoming to treatment is long.

Suramin, a thrombin inhibitor, is a hexasulfonated naphthylurea derivative (Monterio *et al.,* 2004, Fernandes *et al.,* 2007) which was originally synthesized and designed as an antiparasitic agent (Stein, 1993). More recently, suramin has been shown to interfere with the pharmacological effects of some snake venoms such as the myotoxic and paralyzing effects of bothrops toxin-I (Oliveira *et al.,* 2003) and some crotalid venoms (Arruda *et al.,* 2002). In addition, suramin has been reported to inhibit the toxic effects of some PLA_2 neurotoxins like β-bungarotoxin and crotoxin *in vivo* and *in vitro* (Lin-Shiau and Lin, 1999). Suramin significantly delayed the time to paralysis induced by β-bungarotoxin in mice when administered intravenously 30 min before the toxin.

In this study, we have investigated the antagonistic effects of suramin against the lethal effect of the Iranian snake *Echis carinatus* venom in mice.

Materials and Methods

Animals

Adult albino mice (both sexes, weighing 35±5g) were kept under controlled conditions of temperature (26±2°C) and feeding and drinking freely with rodent's normal food and water for one week prior to start the experiments.

Experimental protocols

The ability of suramin (Sigma-Aldrich Chemical Co) to antagonize the lethal effect of *Echis carinatus* venom (Lyophilized crude *Echis carinatus* venom Razi Vaccine and Serum Research Institute, Karaj, Iran) was investigated in five different protocols (A, B, C, D and E) (Table 1). Physiological saline solution has been used to dissolve venom and suramin and route of administrated was intraperitoneal (IP injection).

In protocol A, two groups (A_1 and A_2) were injected with *Echis carinatus* venom at two different doses (A_1, 7 mg/kg and A2, 13 mg/kg, 7 mice in each subgroup). In protocol B, there were three groups (B_1, B_2 and B_3) and suramin was injected into each animal 15 min after injection of venom. In group B_1 (n=8), 15 min after injection of venom (7 mg/kg), suramin 1 mg/kg was injected; in group B_2 (n=8), the dose of suramin was 7 mg/kg; and in group B_3 (n=10), the dose of venom was

increased to 13 mg/kg and suramin was injected at 1 mg/kg (Table 1). Protocol C had two groups (C_1 and C_2). In both groups, suramin and venom were incubated together for 15 min at room temperature (26±2°C) and then injected into animals. In group C_1 (n=8), the doses of both venom and suramin were 7mg/kg, while in group C_2 (n=9), the dose of venom was 13 mg/kg and that of suramin was 1 mg/kg. In Protocol D (n=8), suramin (7mg/kg) was injected into each animal 15 min before injection of venom (7 mg/kg), and, finally, in protocol E (n=6), suramin (1 mg/kg) was administrated into each animal alone.

In all of these protocols, the survival time (in minutes) of each animal after injection of venom was recorded and then statistically compared with groups A_1 and A_2. The animals which remained alive were euthanized after 18 hours for further pathological investigation (the data are not discussed in this paper). Therefore, time to death was recorded as 1080 min for these animals.

Statistical analysis

Statistical analysis was performed by Non-parametric Kruskal-Wallis test and Mann-Whitney *post hoc* test using SPSS-16 (SPSS Inc., Chicago, Illinois). *P* value less than 0.05 was considered significant.

Results

Protocol A, to evaluate the lethal effect of Echis carinatus venom

All mice in group A_1 and A_2 received venom alone. In these groups, the mortality rate was 100%. All animals that received the venom at 7 mg/kg died in average time of 169±32 min, while this time reduced to 53±16 min in group A_2, in which the dose of venom was 13 mg/kg (Table 1 and Fig. 2).

Protocol B, the effects of suramin administered after Echis carinatus venom

In protocol B, all mice in groups B_1, B_2 and B_3 received suramin 15 min after venom. As shown in Fig 2, in group B_1 (which received suramin at the rate of 1 mg/kg, 15 min after 7mg/kg venom), mice died in the average time of 475±133 min, which was significantly different from time to death of animals in group A_1 and A_2 ($p<0.05$). The same results came from group B_2 which received suramin at the rate of 7 mg/kg: the average time to death increased to 620±166 min, which was also significantly different from time to death of animals in group A_1 and A_2 ($p<0.05$). Even when the dose of venom was increased to 13 mg/kg in group B_3 and with suramin at 1 mg/kg, the average time to death (294±131 min) was still significantly different from time to death of animals in group A_1 and A_2 ($p<0.05$) (Fig. 2). Seven animals did not die in these three groups. The mice were euthanized after 18 hours and pathological examinations were done (results not shown here).

Protocol C, effects of pre-incubation of suramin with Echis carinatus venom

In this protocol, suramin was pre-incubated with venom for 15 min prior to IP injection of the mixture into animals. In group C_1, suramin at the rate of 7 mg/kg and 7 mg/kg venom were administrated to animals. The average time to death was 383±107 min, which was significantly different from time to death of animals in group A_1 and in group A_2 ($p<0.05$) (Fig. 2).

In group C_2 (n=9), the dose of suramin was reduced to 1 mg/kg and dose of venom increased to 13 mg/kg. The average time to death was 308±146 min, which was not significantly different from time to death of animals in group A_1, while it was significantly different from time to death of animals in group A_2 ($p<0.05$) (Fig 2). Three animals did not die in these two groups. The mice were euthanized after 18 hours and pathological examination was done (results not shown here).

Protocol D, effect of pre-treatment with suramin on Echis carinatus venom

All mice in group D received suramin at the rate of 7 mg/kg 15 min before administration

Table 1: Summery of experimental protocols.

Protocols	Groups	N, mice	Venom dose mg/kg	Suramin dose mg/kg	Time to death (min)
A	A1	7	7	-	169±32
	A2	7	13	-	53±16
B	B1	8	7	1	474±133
	B2	8	7	7	620±166
	B3	10	13	1	294±131
C	C1	8	7	7	383±107
	C2	9	13	1	308± 146
D	D	8	7	7	200±35
E	E	6	-	1	alive

A: Injected venom only, B: Suramin has been injected 15min after injection of venom, C: Suramin and venom were incubated together 15min before injection into animals, D: Venom has been injected 15min after injection of Suramin, E: Injected Suramin only.



Figure 1: Iranian snake" *Echis carinatus*" known as "*Jafari snake*"; Author's personal collection.

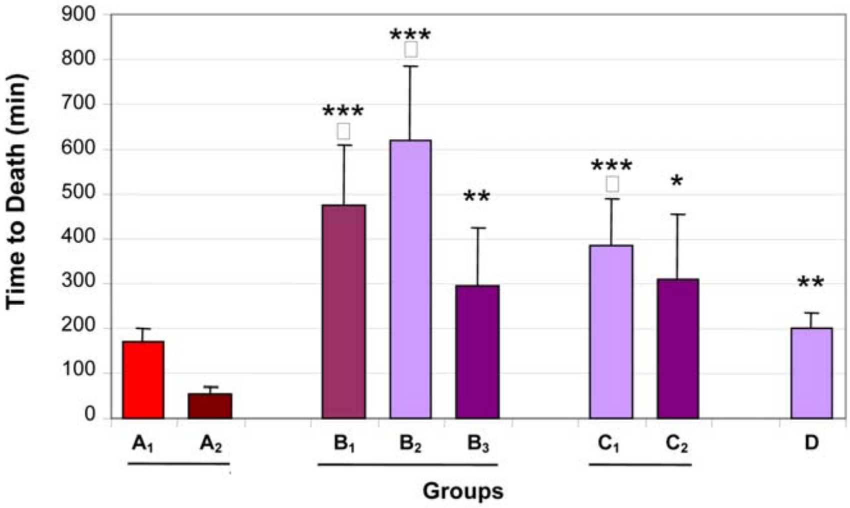


Figure 2: The antagonistic effect of suramin (1 and 7 mg/kg) on time to death of mice after administrated of *Echis carinatus* venom (1, 7 and 13 mg/kg) intraperitonealy (IP). Data are the mean ± SEM of 6 to 10 mice in each group; (●: $P < 0.05$ while compared to group A_1 as control; *: $P < 0.05$, **: $P<0.01$, ***: $P< 0.001$ while compared to group A_2 as controls).

of same dose of venom. The average time to death was 200±35 min, which was not significantly different from time to death of animals in group A_1, although significantly different from time to death of animals in group A_2 ($p<0.05$), (Table 1 and Fig. 2).

Protocol E, to evaluate the effect of suramin alone

Suramin at the rate of 1 mg/kg had no precise effects on animals of this group (n=6) and, as expected, all of them remained alive without any recordable changes (Table 1 and Fig. 2).

Discussion

Echis carinatus is known as the most dangerous snake in the Asia and the Africa with potent anti-coagulation venom. Envenomation by this viper snake is a serious public health issue. This snake is responsible for up to 50,000 deaths each year (Warrell *et al.*, 1977, Backshall 2007).

Immediate treatment of snakebites and getting to an emergency facility as quickly as possible is vital for saving lives. Measures that delay the development of serious symptoms following envenoming could increase the chance of patients accessing medical care and the appropriate antivenin therapy. The results of this study clearly showed that suramin has antagonistic effects against the venom of the Iranian snake *Echis carinatus*. The mechanism of suramin in inhibiting the effects of several different toxins and venoms including *Echis carinatus* venom is not known. Lin-Shiau and Lin, (1999) reported that suramin could delay the neuromuscular paralysing effect of β-bungarotoxin and crotoxin. They concluded that this action of suramin is not related to the effect of this substance on acetylcholinesterase (AChE) activity comparing to neostigmine that blocks AChE activity (Henning *et al.*, 1992, Lin-Shiau and Lin, 1999). It is also reported that suramin inhibited PLA_2 activity of *Bothrops jararacussu* snake venom in a concentration-dependent way (Sifuentes *et al.*, 2008). Since *Echis carinatus* venom contains PLA_2, (Kemparaj *et al.*, 1994), it is possible that suramin counteracts this venom as other PLA_2 venoms. The mechanism of the PLA_2 toxins to induce their effects is not clearly understood, and therefore, it is not easy to explain the mechanism of suramin's inhibitory actions (Fathi, 2001).

There is a possibility that suramin competes with *Echis carinatus* venom components to bind to their acceptors in target cells such as platelets, red blood cells (RBCs), and vascular endothelium.

Another possibility is that suramin directly interacts with toxins. Suramin is a polysulfate anionic compound with rich negative charges that can directly interact with positive charges present in many snake toxins, which are proteins or polypeptides. This may cause a change in configuration of these toxins to delay strong binding to their target sites. It was observed that suramin reduced the pathological damage caused by *Echis carinatus* venom (unpublished results) in mice when injected to the animals by IP route. We conclude that suramin somehow prevents the venom from strongly binding to their acceptors and reduce its effects. Therefore, by reducing the pathological damages such as internal bleeding, suramin slows down the venom effect and in some animals speed up their recovery and prevent them from dying.

In conclusion, the results of our study show that suramin has a protective and inhibitory effect against fatality effect of *Echis carinatus* venom. This drug can postpone the lethal effect of *Echis carinatus* venom, and therefore suramin may have a potential therapeutic application in envenomating.

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اثرات آنتاگونیستی سورامین بر زهر مار جعفری ایران در موش سوری

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چکیده

اکیس کاریناتوس یا مار جعفری به واسطه داشتن زهری با اثرات هموتوکسین، یکی از سمی ترین مارها در آسیا و برخی مناطق آفریقا به شمار می‌آید. گزارش‌ها نشان می‌دهند که داروی ضد تریپانوزوم سورامین قادر است که از اثرات سمی زهر برخی از مارها جلوگیری کند. مطالعه حاضر به منظور بررسی اثرات آنتاگونیستی سورامین بر زهر مار جعفری ایران انجام گرفت. موش‌های سوری بالغ با وزن g ۵± ۳۵ به نه گروه تقسیم شدند (A1، A2، B1، B2، B3، C1، C2، D و E). گروه‌های A1 و A2 (پروتکل A) که به ترتیب زهر را با دوزهای ۷ و ۱۳ mg/kg دریافت کرده بودند پس از ۳۲ ± ۱۶۲ و ۱۶ ± ۵۳ دقیقه تلف شدند. گروه‌های B1 ، B2 و B3 (پروتکل B) با دوزهای متفاوت سورامین ۱۵ دقیقه پس از تزریق زهر درمان شدند. نتایج نشان داد که سورامین زمان تزریق تا مرگ را در گروه‌های B1 ، B2 و B3 در مقایسه با گروه‌های A1 و A2 به طور معنی‌داری افزایش می‌دهد (P<0.05). در گروه‌های C1 و C2 (پروتکل C) سورامین برای مدت ۱۵ دقیقه با زهر انکوبه شده و سپس به موش‌ها تزریق شد. زمان زنده ماندن در این گروه‌ها در مقایسه با گروه A2 به طور معنی داری افزایش یافت. اگرچه که در مقایسه با گروه A1 تنها گروه C1 تفاوت معنی دار نشان می داد (P<0.05). گروه D (پروتکل D) ۱۵ دقیقه پیش از تزریق زهر سورامین را دریافت کردند. زمان تزریق تا مرگ در این گروه در مقایسه با گروه A2 افزایش یافت. در گروه E که به عنوان کنترل تنها سورامین را دریافت کرده بودند تمامی موش‌ها زنده ماندند. نتایج مطالعه حاضر نشان داد که تنها تزریق یک دوز سورامین می تواند اثرات محافظتی در مقابل زهر مار جعفری داشته و زمان مرگ را در حیواناتی که زهر را دریافت کرده‌اند افزایش دهد. احتمال دارد که تزریق های متناوب از دوزهای بالاتر سورامین بتواند از مرگ ناشی از زهر جلوگیری کند.

واژگان کلیدی: سورامین، مار جعفری، زهر، پادزهر، آنتاگونیست